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Creatine kinase MB isoenzyme studies in diagnosis of myocardial infarction

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Serial measurements of the MB isoenzyme of creatine kinase, total creatine kinase, aspartate aminotransferase, and urea stable lactate dehydrogenase have been made in the serum of a series of 139 patients admitted with a diagnosis of suspected myocardial infarction. Serum MB isoenzyme measurements have also been made on postoperative surgical patients and patients with medical disorders known to have caused raised serum total creatine kinase activity.

All these enzymes were abnormal in at least one specimen from all patients with electrocardiographically proved acute myocardial infarction. The magnitude of the MB isoenzyme rise was 2 to 3 times greater than that of any of the other enzymes. The duration of its rise was relatively short. The MB isoenzyme was more specific for myocardial infarction than other enzymes and no increases were found in postoperative patients, except in those after cardiac bypass surgery. The MB isoenzyme seems the most sensitive and specific test for myocardial infarction available, though there are technical problems in its accurate measurement.

The 3 routine enzyme tests most commonly used for the diagnosis of myocardial infarction—namely, aspartate aminotransferase (AST, also known as GOT), lactate dehydrogenase (LD), and creatine kinase (CK)—are not specific for the myocardium: higher concentrations of each of these enzymes may be present in other tissues.

Creatine kinase has 3 principal isoenzymes which are dimers which may contain type B or type M polypeptide chains (Dawson, Eppenberger, and Kaplan, 1965). The BB isoenzyme is found in brain, the MM isoenzyme is the principal component in both skeletal muscle and the myocardium, while the MB isoenzyme is found as a minor component (about 30% of the total CK) in the myocardium and is present in only very small amounts in most skeletal muscles. The MB isoenzyme is, therefore, relatively heart-specific. Though this was reported some time ago (van der Veen and Willebrands, 1966), it is only more recently that a number of investigators have suggested that measurement of serum MB isoenzyme activity may be the most sensitive and specific test for myocardial infarction currently available (Konttinen and Somer, 1972; Roe *et al.*, 1972; Smith, 1972).

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We report here serial studies of the MB isoenzyme of CK in the sera of 139 patients with suspected acute myocardial infarction, of patients with raised total CK activity from causes other than myocardial infarction, and in normal ambulant men.

Patients

Patients with suspected acute myocardial infarction

These comprised 142 patients admitted to the Coronary Care Unit, The Royal Infirmary, Edinburgh, with a diagnosis of suspected myocardial infarction. The load of specimens would have been too great if all consecutive admissions had been included in the study, so allocation to the trial group was on a random, blind basis on arrival at the unit. The patients were classified into the following groups according to electrocardiographic and clinical findings. The enzyme results were not taken into account in making this classification.

1) *Myocardial infarction*—68 patients whose electrocardiograms fulfilled the criteria for 'very probable' myocardial infarction (World Health Organization, 1959)—that is, they showed pathological Q waves, raised ST segment, and subsequent T wave inversion.

2) *Possible myocardial infarction*—14 patients whose electrocardiograms showed fewer diagnostic abnormalities ('possible' myocardial infarction, World Health Organization, 1959), usually ST segment or T wave

changes, or had coexistent or previous changes which precluded inclusion in (1).

3) *'Coronary insufficiency'*—42 patients who had typical cardiac pain unaccompanied by anything more than transient changes in the electrocardiogram.

4) *'Non-infarction'*—15 patients in whom a diagnosis of ischaemic heart disease was thought to be unlikely.

The 139 patients in these groups comprised 109 men and 30 women; 3 further patients were excluded from the study as they died before sufficient data were obtained. Full details of any trauma or excessive exercise before admission were obtained from each patient. Intramuscular injections and resuscitation procedures were also specifically recorded.

Patients without myocardial infarction but with disorders giving rise to raised serum CK activity

These comprised 221 patients. Of these 221 patients, 36 had undergone cardiac bypass operations, mostly for valve replacement, 170 had undergone a variety of non-cardiac surgical operations varying from cystoscopy to major abdominal surgery; 9 were on haemodialysis for acute renal failure; 4 were in grades 3 or 4 coma as a result of barbiturate overdosage; and 2 were 'miscellaneous' patients, one with hypothermia the other with Munchausen syndrome.

Control group

This comprised 132 apparently healthy men, blood donors aged between 30 and 60 years, from whom reference values for the serum enzyme assays were obtained.

Methods

Samples were obtained on admission from patients admitted to the coronary care unit and thereafter at about 6, 12, 18, 24, 36, 48, and 72 hours after admission. Serum was separated within 2 hours of sample collection. Most enzyme analyses were made on the day the sample was received; all others were made within 3 days after storage at +4°C. All specimens with visible haemolysis were rejected.

The following methods were used for serum enzyme activity measurements.

Creatine kinase: Rosalki (1967), using reagents supplied by the Boehringer Corporation, London. Between-batch precision (±1 SD) in the range up to 300 IU/l was 6 IU/l.

Aspartate and alanine aminotransferase: A modification (Smith, Brown, and Taylor, 1970) of the method of Henry *et al.* (1960). Between-batch precision in the range up to 50 IU/l was 1·8 and 2·1 IU/l, respectively, for AST and alanine aminotransferase (ALT).

Urea stable lactate dehydrogenase: Brydon and Smith (1973). Between-batch precision in the range up to 500 IU/l was 20 IU/l.

Rate measurements for the above kinetic methods were made at 37°C at 340 nm using an LKB 8600 reaction rate analyser.

Creatine kinase isoenzymes: These were quantified after separation and staining on polyacrylamide gel slabs (Smith, 1972). Sample volume was 10 µl, serum was diluted if necessary in 40 per cent sucrose to give an activity of 200 IU/l. The stained bands of creatine kinase activity were quantified after densitometry with a Vitatron TLD 100 Flying Spot densitometer by integrating the area under the MM and MB isoenzyme peaks. It was possible to obtain reproducible results only when the MB isoenzyme activity exceeded 10 IU/l, since staining of the isoenzymes was too faint when activity was less than this figure.

The precision of the MB isoenzyme determination was calculated from the results of between-batch repeat analysis of patients' specimens. This indicated an overall coefficient of variation of 15 per cent. The accuracy of the quantification procedure was checked by mixing known proportions of human myocardial MM and MB isoenzymes of CK which had been partially purified by anion exchange chromatography: these mixtures were then analysed by the electrophoretic technique. The results (Fig. 1) indicate that the method is accurate for values below 40 per cent MB isoenzyme within the limits of precision of the technique.

Results

Reference values ('normal range')

The distribution of results for all serum enzymes from the control group was log-normal. The corresponding reference values (Table 1) are 90 per cent confidence limits (5th to 95th centiles) obtained by the relevant graphical parametric methods. From our own previous studies as well as those already published (e.g., Goldberg and Winfield,

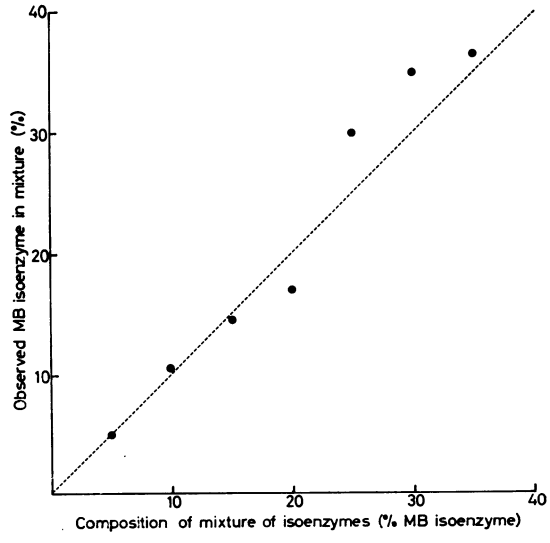

FIG. 1 *Mixtures of known composition of MM and MB isoenzymes of creatine kinase were analysed by electrophoresis and quantified on polyacrylamide gel slabs.*

TABLE 1 *Reference values: healthy ambulant men aged 30 to 60 years*

	Enzyme activity (IU/l) *No.*	*Mean*	*SD*	*Reference range*
Creatine kinase	131	102*	45*	40–200*
Creatine kinase MB isoenzyme	131	Not detectable		0–10
Aspartate aminotransferase	99	20·2	4·9	10–30
Urea stable lactate dehydrogenase	98	203	49	100–300
Alanine aminotransferase	99	19·2	7·8	10–35

*These figures do not include a result of 2460 IU/l.

1974) the following upper reference limits for women were assumed: AST and ALT 25 IU/l, CK 150 IU/l.

The reference values for the MB isoenzyme require further comment. The limit of sensitivity of the present technique was 10 IU/l; below this though MB isoenzyme bands might be visible, they could not be precisely quantified. Therefore samples in which the MB isoenzyme could not be detected, as was the case with all our normal healthy controls, were reported as less than 10 IU/l. A number of independent reports (Yasmineh and Hanson, 1975; Nealon and Henderson, 1975; Henry, Roberts, and Sobel, 1975) have suggested reference ranges of between 0 and 4 IU/l and 0 to 7 IU/l. Probably, therefore, the lower limit of detection by our method—that is, 10 IU/l—is fairly close to the upper reference value for normal men. We therefore accepted this value in this study, though probably the figure is a little high.

Patients with myocardial infarction

In most of these patients (53/68) the initial blood specimen was obtained within 6 hours of the onset of chest pain, and in the remainder specimens were obtained within 24 hours. The activity of each of the serum enzymes was raised in at least one specimen in all 68 patients in this group. The serum levels of the MB isoenzyme differed from the other enzymes in two major respects.

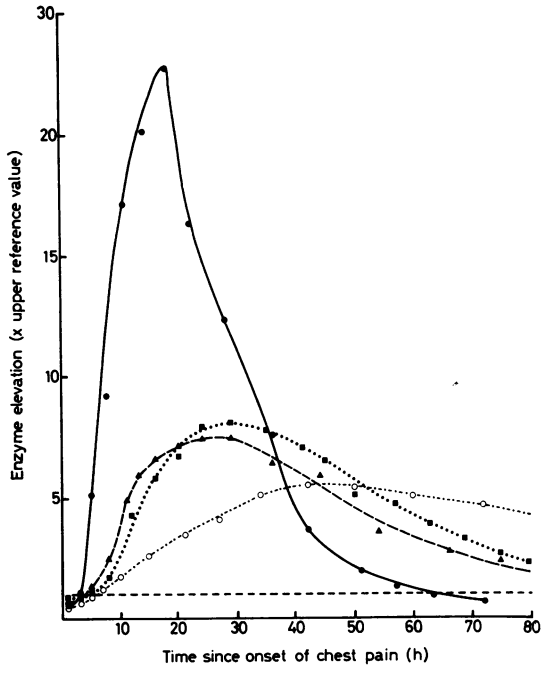

FIG. 2 *Mean serum enzyme activities in group 1 patients at varying intervals after myocardial infarction.* ●——●, *MB isoenzyme;* ▲— — —▲, *creatine kinase;* ■.....■, *aspartate aminotransferase;* o.....o, *urea stable lactate dehydrogenase.*

1) The MB isoenzyme activity rose to higher levels (relative to the upper reference value) than any of the other enzymes (Table 2). For each enzyme the 'peak activity' was the highest measured enzyme activity. With 6-hourly sampling the actual peak may have been missed for the MB isoenzyme in some cases, since the serum levels changed rapidly; much flatter peaks were obtained for the other enzymes.

2) The MB isoenzyme became abnormal earlier, reached its peak activity earlier, and returned to normal earlier than the other enzymes (Table 3, Fig. 2).

The proportion of the total CK contributed by

TABLE 2 *Maximum serum enzyme results in patients with myocardial infarction*

	Upper reference value (IU/l)	*Serum enzyme activity (× upper reference value)* *Mean*	*Range*	*Time of peak activity (h since onset of pain)* *Mean*	*Range*
Creatine kinase	200	8·6	1·5–16	23	9–48
MB isoenzyme	10	22·2	2·8–70	18	8–30
Aspartate aminotransferase	30	8·3	1·3–20	26	11–48
Urea stable lactate dehydrogenase	300	5·7	1·2–14	40	11–69

TABLE 3 *Incidence of abnormal serum enzyme activities at varying intervals since onset of symptoms of myocardial infarction*

Interval since infarction (h)	*No. of specimens*	*CK (MB)*	*% abnormal results CK*	*AST*	*Urea stable LD*
0–6	51	58	28	32	18
7–12	56	98	93	95	78
13–18	53	100	98	98	96
19–24	55	96	94	94	96
25–36	54	94	97	97	98
37–48	50	66	98	98	100
48–72	55	41	85	95	100

CK (MB), MB isoenzyme of creatine kinase; CK, Creatine kinase; AST, Aspartate aminotransferase; LD, Lactate dehydrogenase.

the MB isoenzyme was very variable, both in specimens showing peak MB isoenzyme activity and those showing peak total CK activity. This percentage MB isoenzyme at the MB isoenzyme peak was not related to the magnitude of CK increase (Table 4), to the site of the infarct, to any history of angina or previous myocardial infarcts, or to the age or sex of the patient.

The plasma half-life of the MB isoenzyme was calculated in those patients in whom adequate data were available. Data obtained after enzyme levels had begun to fall were used, on the assumption that by this time significant release of enzyme from the myocardium had ceased. The MB isoenzyme had a mean plasma half-life of 7 hours (range 4 to 12 hours) compared with 18 hours for total CK and 24 hours for AST.

The patient who showed the least pronounced rise in MB isoenzyme activity (28 IU/l) had fairly high activity of CK, AST, and urea stable LD (921, 124, and 1248, IU/l, respectively). It was a feature of these enzyme changes, however, that the enzyme activities remained relatively static throughout the period of 72 hours while the patient remained in the coronary care unit rather than showing the usual peak followed by a decline. This suggests that the patient may have had a series of small extensions to his infarct, causing a much larger rise in CK, AST, and urea-stable LD than in the MB isoenzyme because of the much shorter plasma half-life of the latter.

TABLE 4 *Variability of MB isoenzyme percentage at MB isoenzyme peak*

% MB isoenzyme at MB isoenzyme peak	*No. of patients*	*Mean total CK at MB isoenzyme peak (IU/l)*
Not detectable	0	—
0–4	1	—
5–9	12	1020
10–14	9	1080
15–19	17	940
20–24	13	827
25–29	6	1051
30+	10	1024

Patients with probable myocardial infarction

While electrocardiographic changes were less developed in the 14 patients in this group, most were sufficient, when reviewed with the clinical findings, to warrant a diagnosis of recent intramural infarction. Twelve patients had characteristically raised CK, MB isoenzyme, AST, and urea stable LD results which followed the typical myocardial infarction pattern, and there seems no doubt that this diagnosis was correct in these patients.

A further two patients showed a rise only in the MB isoenzyme; the other enzymes showed small rises within the reference range. One of these patients developed unequivocal electrocardiographic and enzyme evidence of infarction 4 days later; the other also developed highly suggestive sequential electrocardiographic changes.

Patients diagnosed as having myocardial ischaemia

There were 42 patients in this group. Ten of them showed enzyme changes characteristic of myocardial infarction with a rise in all cardiac enzymes. The electrocardiographic findings in these 10 patients were variable: 1 was normal, 5 showed evidence of previous infarction but no fresh changes other than transient ST segment rise, 6 showed transient ST segment changes, 2 had arrhythmias, and 1 showed digitalis effects; more than 1 of these abnormalities were sometimes present. In 20 patients all enzyme results were normal. In the remaining 12 patients enzyme rises were present, but in the light of clinical and laboratory data these enzyme changes were thought to be the result of factors other than myocardial infarction. Further details are given in Table 5.

TABLE 5 *Abnormal enzyme results in patients with chest pain from causes other than myocardial infarction*

Diagnosis	*Enzyme results (IU/l)* *MB isoenzyme*	*CK*	*AST*	*Urea stable LD*
MB isoenzyme mainly affected				
Paroxysmal atrial fibrillation	45	260	50	420
Pericarditis	40	N	N	N
Unexplained chest pain	28	N	N	N
CK mainly affected				
Unexplained (? myositis)	N	664	68	320
Following dental extraction	N	460	N	N
Unexplained	N	340	N	N
Adams-Stokes attack	N	300	N	350
AST mainly affected				
Unexplained*	N	N	420	N
Heart failure*	N	N	230	340
Alcohol excess*	N	N	100	N
Heart failure*	N	N	90	630
Unexplained*	N	N	69	N
Unexplained*	N	N	46	N
Alcohol excess	N	N	41	N
Urea stable LD mainly affected				
Haemolysis	N	490	80	3700
Heart failure	N	N	34	520
Unexplained	N	N	N	340

*These patients all showed increased serum alanine aminotransferase activity.
N—within the reference range.

Patients diagnosed as having 'non-cardiac' disorders

In 10 of these 15 patients all enzyme results were normal; in the remaining 5 one or more enzyme results was abnormal. These patients are also included in Table 5.

After reviewing all the evidence, 92 of the 139 patients were thought to have had a myocardial infarct. In 90 of these all 'cardiac enzymes' were raised, in 2 only the MB isoenzyme was raised. Seventeen of the 47 patients thought not to have had myocardial infarcts had raised enzyme activities. This important group will be considered in more detail, since such cases most often cause diagnostic difficulty and consequently misdiagnosis.

In only 3 cases was a rise in the MB isoenzyme not attributed to myocardial infarction. Two of these were readily explicable in terms of release from myocardium—patients with paroxysmal tachycardia and pericarditis. The remaining patient, a man of 32 with a history of alcoholism, presented with chest pain which clinically did not seem to be of cardiac type. The MB isoenzyme activity (28 IU/l) was the only abnormal enzyme finding, and the electrocardiogram was normal.

Of the 6 patients with raised serum CK activity not caused by infarction, it was impossible to ascertain the cause with confidence in 3, though 2 of them had had intramuscular injections. However, in these 3 patients and in the patients with rises after dental extraction and an Adams-Stokes attack the MB isoenzyme was absent from serum. Thus, 5 out of 6 patients in this group with raised total CK had normal MB isoenzyme findings—a feature that highlights the value of isoenzyme studies in these anomalous cases.

Aspartate aminotransferase seemed the least specific of the enzyme tests in that 11 patients had increases unrelated to myocardial infarction. Most of these patients probably had hepatocellular damage (2, for example, had been drinking very heavily before admission); in 6 patients the ALT results were valuable, as large increases in this enzyme were also present. Thus, if ALT results are taken into account in interpreting AST findings the overall specificity of AST is probably not much different from CK and urea stable LD.

The urea stable LD results suggest this is a fairly specific test for myocardial damage. However, a number of specimens showing minor degrees of haemolysis had to be rejected. Experience has shown that even such minor degrees of haemolysis, which in a busy routine laboratory may often be missed, can cause significant increases in urea stable LD activity.

Postoperative patients (Table 6)

a) Cardiac bypass Most of these had received valve replacements, with periods on bypass usually of between 2 and 4 hours. All 36 patients had raised

TABLE 6 *MB isoenzyme findings in patients with diagnosis other than myocardial infarction*

Category	*No. of patients*	*No. with raised CK activity*	*Range of CK activities (IU/l)*	*MB isoenzyme in patients with raised CK (IU/l)*			
				<10	*10–49*	*50–99*	*>100*
Cardiac bypass	36	36	240–2300	24	6	5	1
Surgical postoperative	170	46	up to 2500	46	0	0	0
Acute renal failure	9	6	up to 940	6	0	0	0
Self-poisoning	4	3	up to 960	3	0	0	0

CK levels but in only 12 was the MB isoenzyme detectable. In only one case, however, was the MB isoenzyme percentage greater than 10, suggesting that most of the CK in the serum of these patients had arisen from skeletal muscle.

b) Other postoperative patients We have demonstrated only 46 out of 170 patients with raised serum CK after operation, though this figure should not be regarded as necessarily indicating the true incidence of such increases, since a single random specimen was obtained from each patient and therefore serum CK increases which may have been detectable with serial blood sampling would have been missed. No patient showed an MB isoenzyme in his serum.

Other disorders giving rise to raised serum CK activity (Table 6)

None of the patients with acute renal failure or barbiturate overdosage showed any MB isoenzyme activity in their serum. One patient with hypothermia had a serum CK of 5100 IU/l; no MB isoenzyme was present in his serum. Another patient was admitted to the coronary care unit (but not included in the trial) complaining of severe chest pain. His electrocardiogram was abnormal but showed no evidence of recent infarction: however, his serum CK was 3400 IU/l; AST and urea stable LD were normal. No MB isoenzyme was present in his serum. Further inquiries at other hospitals in South Scotland indicated that he had visited several of them before his arrival in Edinburgh, with a similar story in each case. It is not clear why this man with the Munchausen syndrome had such a high serum CK.

Discussion

The merits of any new test must be compared with the known advantages and disadvantages of existing tests. Firstly, clinical considerations should be paramount, though methodological and other factors may affect the final decision whether to put a test into routine diagnostic use.

In many cases enzyme tests have to confirm or refute a diagnosis of myocardial infarction. The information may be required for a decision on immediate management—for example, whether to keep a patient in a coronary care unit—and it may be of importance to long-term management and prognosis. Detailed analysis of enzyme results may also give a good index of infarct size (Sobel *et al.*, 1972). Indeed, creatine kinase results obtained in the first few hours after admission have been used to predict infarct size (Shell *et al.*, 1973). These authors now claim that by using such studies they can assess the efficiency of certain drugs in reducing the size of infarcts (Shell and Sobel, 1974).

If the enzyme findings are taken into account in classifying the patients into 'infarction' and 'non-infarction' groups 92 of our 139 patients probably had infarcts. Though there are differences in the overall sensitivity of serum CK, AST, and urea stable LD they are relatively minor, since in 90 of the 92 cases all 3 enzymes were abnormal. In the remaining 2 cases only the MB isoenzyme was abnormal. Taking this evidence in conjunction with the fact that the average magnitude of MB isoenzyme rise was at least 2 or 3 times as great as that of the other enzymes, there seems little doubt that MB isoenzyme measurements are appreciably more sensitive indices of myocardial damage than enzyme tests hitherto available.

It may be questioned whether quantitative MB isoenzyme assays are necessary, since in the present study the combination of total CK (or AST) and a qualitative assessment of the MB isoenzyme was usually sufficient for the diagnosis of myocardial infarction. There are three main reasons why such measurements could be of use. Firstly, small amounts of myocardial damage could be detected if a more sensitive assay technique could be developed for routine use. Secondly, MB isoenzyme measurements may, by showing a secondary rise, indicate the occurrence of an extension to an existing infarct not detected by the other enzymes because of their slower return to normal levels. Thirdly, because of its inherent specificity for myocardium, MB isoenzyme measurements should be especially valuable in the prediction and measurement of infarct size. We have found, however, that the MB

isoenzyme contributes such a variable proportion of total CK (Table 4) that estimates of infarct size based solely on MB isoenzyme assay may be unreliable, at least until the precision of measurement of MB isoenzyme activities approaches that obtainable for other enzymes.

The lack of specificity of enzyme tests is probably of more concern than any lack of sensitivity. In this respect, MB isoenzyme measurement clearly stands out as superior to any other tests both from the results reported here and those obtained elsewhere (Wagner *et al.*, 1973; Konttinen and Somer, 1973). Whereas CK, AST, and urea stable LD may individually lack specificity, if the results from all three assays are considered together perhaps with the addition of ALT the overall specificity of the group is much improved and is likely to be equal to if not better than that of the MB isoenzyme. The MB isoenzyme should not, however, be regarded as truly specific for myocardium. In the present study the MB isoenzyme was raised in the serum of one patient (an alcoholic) who almost certainly had no myocardial disease: one of us has previously described an apparently healthy man with a serum CK of 400 IU/l who had an increased serum MB isoenzyme (Smith, 1972). The isoenzyme may also be raised in the serum of patients with muscular dystrophy (Goto, Nagamine, and Katsuki, 1969).

Although few difficulties are likely to arise with raised serum enzyme activities in renal disease and as a result of barbiturate overdosage, problems in the immediate postoperative period are much more common. In general, AST, CK, and urea stable LD may be expected to be raised after any surgical operation of any magnitude; this invalidates their use of the detection of postoperative myocardial infarction. It seems likely that the MB isoenzyme measurements may be very valuable in these circumstances since non-cardiac surgery is not accompanied by rises in serum levels of the MB isoenzyme.

Probably the major clinical restriction of the value of MB isoenzyme measurement lies in its short duration of rise. Though it becomes raised before other enzymes this is only a matter of an hour or so and probably not of major importance, whereas the fact that in many cases activities have returned to normal within 36 to 48 hours after the onset of pain may sometimes limit its value. On the other hand, this feature does allow detection of reinfarction or extension of an infarct. Presumably the rapid fall in MB isoenzyme activity is merely an expression of its short half life in plasma (about 7 hours).

In contrast to the relatively minor clinical drawbacks to MB isoenzyme usage there are technical difficulties concerned in its accurate quantification. At present ion exchange chromatography or electrophoresis is used most widely. Most methods are tedious or imprecise or both but simplified techniques are now becoming available, sometimes as commercial reagent kits. Our experience with one such kit (Corning Eel electrophoresis system[1]) suggests that reasonably accurate and precise results for MB isoenzyme activity may be obtained within an hour or so of receipt of the specimen.

Our data from the present study suggest that MB isoenzyme measurement is 1) the most sensitive test for myocardial infarction currently available provided blood samples are taken 12 to 30 hours after the onset of pain; 2) the single most specific test for myocardial infarction; and 3) the only test that materially aids the diagnosis of myocardial infarction in patients in whom there are other causes —for example, surgery or trauma—for generalized enzyme release from damaged tissues (Dixon *et al.*, 1973). We think that these are sufficient reasons to justify the more widespread use of MB isoenzyme measurement.

[1] Corning-Eel, Evans Electroselenium Ltd., Halstead, Essex, England.

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